Radiotracer imaging in PD

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1

Introduction

Aim of this thesis

The main aim of the thesis is to study radiotracer uptake in the "presynaptic" dopaminergic system of the basal ganglia in order to assess its use in Parkinson's disease. The topics addressed here are:

a) the association between the cerebral dopaminergic system and clinical motor signs
b) the possibility to monitor neuroprotection
c) early clinical diagnosis.

The uptake of two well-known tracers namely FP-CIT (SPECT) and F-DOPA (PET) in patients with several stages of Parkinson's disease and in healthy volunteers were compared. Both primate MPTP and human PET or SPECT scans in addition to clinical motor assessments constitute the basis of this thesis.
We also studied the effects of a presumed neuroprotective agent on radiotracer uptake in the animal model and correlated this with motor behaviour and post-mortem histopathological findings.

1. Parkinson's disease

Parkinson's disease is a common neurodegenerative disorders, mainly affecting the elderly. It is a slowly progressive disorder in which progression is believed to be fastest in the beginning of the disease[52]. When symptoms and signs become evident, already probably 30% or more of the dopaminergic cells have been lost and endogenous striatal dopamine levels may be only 5-15% of normal[39]. Clinical symptoms consist of the classic trias of resting tremor, rigidity and bradykinesia. Next to these motor symptoms, also non-motor symptoms like depression, hallucinations and cognitive disorders exist. In chapter 1, a more extensive description of Parkinson's disease can be found.
Until so far, only symptomatic treatment is available. However, after some years, this treatment often becomes less effective. Unfortunately, there is no therapy available yet to stop or even delay the underlying process. Neuroprotection can be defined as an intervention that slows or stops the progression of neuronal degeneration, interfering with the underlying aetiology.

1.a.Pathophysiology in humans

The specific aetiology of Parkinson's disease (PD) is not known yet, but it is likely to be the result of the cumulative effects of environmental and genetic factors. Main risk factors for developing PD include advancing age[16] and positive family history[24, 53], suggesting that it is an age-dependent genetic disorder, at least in a subset of patients. Epidemiological studies suggest some factors may increase the risk of developing PD. During the last

decades several studies have been published, some suggesting environmental factors, others genetic factors to be the main cause of PD. Probably PD results from an interaction of (multiple) genetic mutations and environmental toxins: genetic factors may make an individual person more vulnerable to environmental factors. This is known as the double-hit hypothesis[29].

An increased risk for the development of PD has been found in association with environmental factors like exposure to well water drinking, herbicides (e.g. paraquat), pesticides (e.g. rotenone), industrial chemicals, farming and living in a rural environment[71]. Trace metals, cyanide, lacquer thinner, organic solvents, carbon monoxide and carbon disulphide have also been associated with an increased risk of developing PD[72]. However, post mortem analysis of brains of parkinsonian patients revealed no specific toxin. Special attention has been focused on the neurotoxin 1,2,3,6-methyl-phenyl-tetrahydroyridine (MPTP). MPTP, used in the drug scene, is a contaminate of the production of a synthetic meperidene derivate. After taking MPTP, drug addicts developed a syndrome, which clinically and pathologically closely resembled idiopathic PD[40]. Although many mechanisms have been proposed to be involved in the process of progressive cell death in Parkinson's disease, like oxidative stress, glutamate excitotoxicity, free radical damage, mitochondrial (complex I) dysfunction and inflammatory processes or proteasomal dysfunction, the exact mechanisms responsible for the process are still unknown[29, 39, 59]. As we have studied the possible neuroprotective effect in PD of an anti-apoptotic agent, we will focus now on the role of apoptosis in the pathogenesis of Parkinson's disease.

1.a.1. Apoptosis in pathogenesis of Parkinson's disease

Parkinson's disease is a slowly progressive disorder characterised by loss of dopaminergic neurons in the substantia nigra and degeneration of nigrostriatal pathways, resulting in a decrease of striatal endogenous dopamine concentration.

Although the mechanisms of cell death in PD are still unknown, general belief is that the neuronal death in the pars compacta of the substantia nigra is apoptotic[12, 29]. However, the exact role of apoptosis remains unknown and necrosis may be involved as well. Apoptosis is a form of programmed cell death and is to a certain extent a normal physiologic process in dopaminergic neurons. It is characterised by nuclear chromatin condensation, intact cytoplasmic membranes, DNA fragmentation and cell shrinkage without inflammatory processes.

Some studies have found evidence for apoptosis in PD, while others were not able to confirm this. In 1996 Mochizuki et al found, using in situ DNA end labelling, cells displaying DNA cleavage in the substantia nigra of 4 patients with PD, without apoptotic morphology, such as chromatin clumps[50]. However in situ DNA end labelling is prone to false-positive detection of apoptosis. One year later, Anglade detected typical features of

apoptotic neurons in the substantia nigra of 3 patients with PD using electron microscopy, like condensed chromatin in the nucleus of neurons containing neuromelanin and intact cytoplasmic membranes[4]. Using fluorescent probes specific for both DNA cleavage and chromatin clumping, Tatton was able to confirm positive staining of melanized neurons in the pars compacta of the substantia nigra[73]. However, all of the studies mentioned are limited by the small numbers of patients studied and by the lack of suitable control material. By the absence of any data from age-matched normal brains, the significance of these findings for the aetiopathology of PD remains unclear. More recent studies have demonstrated increased numbers of cells that express markers of apoptosis, with individual nuclei staining positively for DNA fragmentation and chromatin clumping. Increased expression of cell signals associated with apoptosis, including BAX, caspase-3 and nuclear translocation of glyceraldehydes phosphate dehydrogenase have been detected in PD[27, 59, 74]. Increased expression of the pro-apoptotic factor p53 in nigral neurons of PD patients have been observed[54]. Those studies suggest that apoptosis could account for some of the cell death occurring in PD [59, 64]. Apoptosis may be the primary form of cell death, but it cannot be excluded that it may be secondary to other forms of cell death which trigger apoptosis. By inhibiting apoptosis, the underlying progressive neurodegenerative processes of PD might thus be blocked, resulting in effective neuroprotection.

1.b. Animal models

After the discovery of MPTP as a neurotoxin with particular affinity for the dopaminergic neurons, an animal model using this compound, has been developed. The MPTP-model is so far one of the best experimental model for the production of parkinsonism in animals and reproduces all its cardinal features of idiopathic Parkinson's disease like rigidity, bradykinesia and sometimes tremor[2, 9, 19, 40]. A number of analogues of MPTP can cause similar damage to the nigrostriatal system[26].

Other agents used in animals to cause parkinsonism are 6-OHDA, paraquat, rotenone, lipopolysaccharide[49] and proteasomal inhibitors like lactcystin and epoxomicin[45]. Also genetic models of Parkinson's disease exist, in which animals have been genetically manipulated. All those compounds can be administered to various animals, but are most widely used in rodents and in non-human primates. The advantage of the latter above other animal species is that those animals are more similar to the human in both brain pathology and symptomatology. Therefore we used in our studies, the MPTP-treated monkey.

1.c. Neuroprotection

Neuroprotection can be defined as an intervention that slows or stops the progression of neuronal degeneration and seeks to interfere with the basic pathogenetic mechanism of nigral cell death. We have investigated the possible neuroprotective effect of TCH346 in MPTP-treated monkeys, see chapter 4.

1.c.1. MAO- inhibitors: selegiline

One of the first agents thought to be neuroprotective was the propargylamine selegiline (also known as Deprenyl), a selective irreversible inhibitor of mono-amine-oxidase, type B (MAO-B). Because of its ability to block the MAO-B metabolism of dopamine, selegiline was thought to increase striatal dopamine. In MPTP-treated animals, conversion of MPTP to MPP+ can be inhibited by blocking MAO-B, thus preventing the development of nigrostriatal degeneration and parkinsonian symptoms in experimental animals[41]. Thus MAO-B-inhibitors may prevent the conversion of a protoxin to a toxin, and could possible be neuroprotective. Knoll reported that selegiline extended lifespan in rats by 50% [35]. Birkmayer et al tested selegiline in PD and reported modest antiparkinsonian benefits, a reduction in motor fluctuations, longer survival and less disability when selegiline was combined with levodopa[7, 8]. In 1987 a double-blinded, placebo-controlled, multicenter trial, the DATATOP-study (Deprenyl and Tocopherol Antioxidative Therapy Of Parkinsonism) was started to determine if deprenyl 10 mg/day or tocopherol 2000 IU/day, administered to untreated patients with early PD, would slow disease progression and prolong the time until levodopa therapy was necessary. In this study, 800 patients were enrolled[1]. According to this study, selegiline significantly delayed the development of disability requiring levodopa therapy. However, after drug withdrawal a worsening of the motor scores was observed. This indicates a symptomatic effect instead of a neuroprotective effect. Possible neuroprotective effects may have been masked by these symptomatic effects.

There was no evidence of sustained advantages of selegiline, as measured by complications of L-dopa therapy or duration of life[36, 58, 68]. However, in the patients treated with selegiline, more cardiovascular adverse events and mortality occurred compared to the placebo-treated patients. This increase in adverse events can be explained by the amphetaminergic metabolites of selegiline. Because of the lack of proof for a neuroprotective effect and the amphetaminergic metabolites, selegiline is no longer eligible as a possible neuroprotective agent for Parkinson's disease.

1.c.2. Apoptosis inhibitors: TCH346

Another propargylamine without the toxic metabolites amphetamine and methamphetamine is CGP 3466B (dibenzo[*b,f*]oxepin-10-ylmethyl-methyl-prop-2-ynyl-amine), also known as TCH346. Unlike deprenyl, TCH346 does not inhibit MAO-B and cannot be metabolised into amphetamine and metamphetamine.

TCH346 has been tested in a wide variety of cellular and animal models of PD and exhibits neurorescuing properties qualitatively similar to, but about 100-fold more potent than those of (-)-deprenyl[79, 80]. TCH346 does not inhibit MAO-B, but interacts with GAPDH (glyceraldehyde-3-phosphate dehydrogenase). GAPDH is a glycolytic enzyme. Beside its role as enzyme in the glycolysis, GAPDH has been found to play a critical

role in apoptosis. Post-mortem analyses of brains of parkinsonian patients show nuclear accumulation of GAPDH in the substantia nigra (SN), suggesting that GAPDH plays a role in the neurodegenerative processes or apoptosis in PD. Normally GAPDH is confined to cytosol by RNA. During apoptotic death, GAPDH is overexpressed. This results in an increased amount of GAPDH, accumulating in the nucleus, accompanied by a decrease of affinity of GAPDH for RNA. Accumulation of GAPDH in the nucleus may result in malfunctioning of the cell, leading to increased glycolysis and eventually cell death. GAPDH exists in two forms: a dimeric and a tetrameric form. GAPDH can only exert its apoptotic function in its tetrameric form and not in its dimeric form. It is possible that GAPDH dimer cannot accumulate in the nucleus. TCH346 blocks the action of GAPDH by preventing accumulation of GAPDH in the nucleus of the cell and / or by converting GAPDH from its tetrameric form to a dimeric form[13].

1.c.2-1 Animal studies

The compound is not only able to rescue dopamine neurons in vitro from death induced by apoptotic stimuli[37], but also has promising effects in rodent models of PD. Doses of 0.0014-1.4 mg/kg TCH346 given twice daily for 18 days have neuroprotective effects in mice. In addition, the compound prevents nigral degeneration and motor symptoms induced by low doses of 6-OHDA in the rat model of PD[3]. We have extended the rodent studies to an animal more similar to the human in both brain organisation and motor function, in order to analyse the neuroprotective effects of TCH346 in MPTP-treated rhesus monkeys (see chapter 4). The MPTP monkey model reproduces virtually all the classic behavioural, cognitive, biochemical and histological changes that occur in PD. We have also studied the correlation between behaviour parameters and degeneration of the nigrostriatal dopamine systems established with [^{18}F]-DOPA positron emission tomograph (PET) scans in this animal model (see chapter 3).

1.c.2-2. Human studies

Because of the positive results of the neuroprotective effect of TCH346 in MPTP-treated monkeys, in this thesis described in chapter 4, this compound has also been tested in the human setting.

In a double-blind, randomised, placebo-controlled trial, around 300 patients with de novo PD with Hoehn and Yahr (H&Y) stage of 2 or less and disease duration of less than 1 year were included[60]. They were randomly assigned to TCH346 in 3 different doses or placebo. Time until symptomatic treatment was needed was the primary outcome measure and changes in Unified Parkinson's Disease Rating Scale (UPDRS; total, part II and part III) and Parkinson's Disease Questionnaire (PDQ) 39 formed the secondary outcome measures. Patients received the study drug for 12 to 18 months followed by a 4 week

withdrawal period, or until symptomatic treatment was needed. However, in all the 4 treatment groups an equal percentage of patients needed symptomatic treatment. Also no significant difference was found in secondary outcome measures between the 4 treatment groups. So it can be concluded that TCH346 is not neuroprotective in patients with PD[60].

1.c.2-3 Contradictory outcomes

Although the animal studies showed a neuroprotective effect of TCH346 in several animal models, this could not be detected in the human setting. Several factors can be mentioned to explain this difference in efficacy (see also Chapter 8)[60, 78].

- the MPTP- treated animal model does not reflect the pathogenesis of PD correctly.
- inaccurate doses of TCH346 have been administered to patients with PD.
- PD had already progressed too far in the included patients
- the follow-up period of 1 year was too short to be able to notice subtle neuroprotective effects
- the chosen end-points were not sensitive enough to detect subtle changes

The contradictory outcomes between animal studies and human studies underscore the need for the development of a better progressive animal model, reflecting more accurately pathogenetic mechanisms of PD[60, 65].

1.d. Early diagnosis

It is of interest to be able to diagnose PD in the early phases of the disease. Neuroprotective treatment, if available, should be given as soon as possible after the onset of signs and symptoms, as progression is probably fastest in the beginning of the disease[52] and therefore neuroprotection is most effective in those early phases.

In the beginning of the disease, diagnosing PD correctly on clinical signs and symptoms can be difficult. The motor symptoms can be subtle in the first phases of PD. More importantly, the clinical features of PD may occur in other neurodegenerative disorders as well, like Multi System Atrophy (MSA), Progressive Supranuclear Palsy (PSP) or Cortico Basal Degeneration (CBD). Differentiating idiopathic PD from these other forms of parkinsonism has therapeutic and prognostic impact.

A non-movement disorder neurologist may misdiagnose up to 25% of cases with established parkinsonism of all sorts when compared with post-mortem pathology[31, 48]. Movement disorders specialists are more often able to diagnose idiopathic PD correctly when all the relevant clinical information is obtained: the positive predictive value of the clinical diagnosis PD was under those circumstances 98,6%[30]. These studies refer to advanced PD patients. No data are available to assess the situation in early, de novo, PD patients who on clinical grounds are likely to have indeed PD, but in whom this as yet could not be confirmed by response to medication or by the time course. Therefore, to facilitate

diagnosing PD correctly in the above mentioned circumstances, auxiliary examinations are needed.
Several non-motor signs and symptoms in PD have been tested for their utility in diagnosing PD in early (preclinical) phases, like olfactory dysfunction[17, 18, 81], disturbances in mood and personality (e.g. depression)[15, 28, 51, 84], visuomotor control abnormalities and subtle neurocognitive dysfunctions[11, 14, 20]. However, none of these signs and symptoms are sensitive nor specific for PD[6, 83].
Conventional imaging techniques of the brain, such as CT or MRI scans, are also not useful for diagnosing PD early, because the brain structure is usually not greatly altered in early PD and can not positively document dopaminergic biochemical activity.

2. Functional imaging

Another entrance for auxilliary methods in diagnosing PD is formed by visualising the underlying biochemical abnormalities in PD. At the time of diagnosis probably 30% or more of dopaminergic neurons are lost and endogenous striatal dopamine levels are estimated to be 5-15% of controls[39]. The preclinical phase is thought to be between 3.1[52] to 6.5 years[57]. This implies that patients can possibly be identified in a preclinical phase of the disease by assessing the biochemical changes. Striatal activity of aromatic aminodecarboxylase and the density of dopamine transporters is decreased in patients with PD. Radiotracer neuroimaging techniques using Positron Emission Tomography (PET) or Single Photon Emission Computed Tomography (SPECT) can be helpful to visualise and measure striatal dopaminergic activity.
Several radiotracers are developed to investigate the dopaminergic system and most of them can be divided in “presynaptic” and “postsynaptic” tracers. Presynaptic tracers are able to measure striatal activity of aromatic aminodecarboxylase (^{11}C-DOPA, ^{18}F-DOPA, ^{18}F-FMT) or the density of dopamine transporters (^{11}C-CFT, ^{11}C-MP, ^{123}I-FP-CIT or ^{123}I-β-CIT). Postsynaptic tracers are able to quantify the postsynaptic D2 receptor binding: ^{11}C-raclopride, ^{18}F-NMSP, ^{18}C-FLB457, ^{11}C-SCH23390 or ^{123}I- IBZM. Finally, the vesicular monoamine transporter can be quantified by the use of ^{11}C-DTBZ. F-DOPA PET has been regarded as the gold standard for the assessment of presynaptic dopaminergic integrity in vivo, in part because it has been in use for many years and has been extensively studied[55]. F-DOPA PET was the first developed tracer to visualize the nigrostriatal dopaminergic system in vivo[23]. However, its use is limited by the restricted availability of PET instruments, the need for a cyclotron and the difficult production of F-DOPA. FP-CIT SPECT is easier available and can therefore form an alternative for F-DOPA PET. We compared the use of FP-CIT and F-DOPA by means of respectively SPECT and PET in patients with Parkinson’s disease and in healthy volunteers for diagnosing Parkinson’s disease in an early phase in chapter 6, and the correlation between uptake measures and clinical symptoms in chapter 5.

2.a. Positron Emission Tomography

Both PET and SPECT use radioactive tracers: compounds with a radioactive atom attached to a molecule. Radioactive atoms decay, emitting gamma rays and nuclear particles. PET uses metabolically active compounds labelled with short-lived radioactive tracer isotopes, which decay by emitting a positron. After travelling up to a few millimetres, the positron encounters and annihilates with an electron, causing two gamma photons to be emitted in opposite directions. The tomographic image is formed by recording two 511 keV photons emitted in positron decay with a circumferential array of radiation detectors. Since these photons are simultaneous emitted at approximately 180 degrees to each other, it is possible to localize their source along a straight line of response. After processing these data through a conventional reconstruction algorithm, tomographic images of the tracer accumulation in the tissue can be formed.

2.b. Single Photon Emission Computed Tomography

SPECT is similar to PET in its use of radioactive tracer material and detection of gamma rays. In contrast with PET, however, the tracer used in SPECT emits gamma radiation which is measured directly. Also the level of energy of the emittors used in SPECT is different from those used in PET. ^{123}I is used for FP-CIT SPECT which emits gamma rays of 159keV. SPECT imaging is performed by using a gamma camera to acquire multiple 2-D images from multiple angles. A computer is then used to apply a tomographic reconstruction algorithm to the multiple projections, yielding a 3-D dataset. This dataset may then be manipulated to show thin slices along any chosen axis of the body, similar to those obtained from other tomographic techniques. To acquire SPECT images, the gamma camera is rotated around the patient and projections are acquired at defined points during the rotations.

2.c. F-DOPA PET

PET scans using 6-[^{18}F]-fluoro-L-3,4-dihydroxyphenylalanine (F-DOPA) enable measurement of striatal levodopa decarboxylase activity, thereby estimating the rate of enzymatic decarboxylation of F-DOPA to F-dopamine, and trapping of F-dopamine in synaptic vesicles. Striatal F-DOPA uptake does not measure the endogenous dopamine concentration. It correlates with dopamine cell counts measured in post mortem specimens[69]. It is possible to discriminate patients with PD from the healthy population by means of F-DOPA PET. Several studies have reported a decrease in striatal F-DOPA uptake in PD compared to healthy controls, more pronounced in the putamen than in the caudate[5, 22, 43, 44, 63]. However, restricted availability of F-DOPA and PET instruments limit its use in routine clinical practice. It has been suggested that in the very beginning of this disease, levodopa decarboxylase activity is upregulated although this never has been proven. Such factors, however, may influence the sensitivity of this technique for diagnosing defects in the nigrostriatal system.

2.d. FP-CIT SPECT

Uptake of tracers with a high affinity for the dopamine transporter (DAT) can be measured using PET and also SPECT. Dopamine transporters, localised on dopaminergic nerve endings, participate in the reuptake mechanism of dopamine into presynaptic terminals and are modulated by concentrations of endogenous dopamine[25]. Decrease of transporter density in the striatum has been associated with PD[33, 56]. DAT imaging can therefore be used as a marker for the relative degree of malfunction or loss of dopaminergic nerve endings. A selective and potent DAT imaging agent is [^{123}I] N-ω-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl) nortropane (FP-CIT). SPECT imaging with FP-CIT produces a high target to background ratio. Several studies have demonstrated that striatal FP-CIT uptake is reduced in patients with PD compared to healthy controls[10, 32, 34, 46, 47, 66, 67, 75, 76]. FP-CIT SPECT is, in contrast to F-DOPA PET, widely available and cheaper than F-DOPA PET. However, spatial resolution is lower in FP-CIT SPECT scans than in F-DOPA PET scans, resulting in poorer quality of the SPECT images. Another disadvantage of FP-CIT is the disability to continue medication known to interfere with DAT, like methylphenidate, benzatropine, bupropion, mazindol, phentanyl, sertraline and cocaine-derivates, before scanning, while it is not necessary to stop most medication before F-DOPA PET scanning. Only catechol-O-methyl-transferase (COMT)-inhibitors may influence the outcome of a F-DOPA PET scan. Finally, the duration of a FP-CIT SPECT scan with a regular double-headed gamma camera is longer (approximately 45 minutes) than the duration of a 3-D F-DOPA PET scan (approximately 6 minutes). This long duration of scanning can be a major disadvantage of FP-CIT SPECT scanning in parkinsonian patients, especially when they suffer from tremor or dyskinesia.

2.e. Other radiotracers

Next to the above mentioned presynaptic dopaminergic tracer methods, postsynaptic dopaminergic tracers have been developed, like ^{11}C-raclopride for PET or ^{123}I- IBZM for SPECT. Decreased binding of postsynaptic dopaminergic tracers suggest the presence of other forms of neurodegenerative disease than Parkinson's Disease. However, those tracers can not be used in clinical practice to confirm the diagnosis of Parkinson's Disease.

Another alternative is formed by FDG-PET scanning. This method is nowadays frequently used in oncology. By recognition of the pattern of brain metabolism, FDG-PET can be used in clinical practice for differentiation between different forms of parkinsonism, like MSA, PSP and CBD[21]. ^{99m}Tc-ethyl cysteinate dimer (ECD)-SPECT is sometimes used to differentiate between the different forms of neurodegenerative disorders as well by measuring regional cerebral blood flow[85]. However, the spatial resolution of SPECT is too low for an easy discrimination between the various forms of parkinsonism[42].

Outline of this thesis

In **chapter 1** the pathophysiology of Parkinson's disease in humans and in animal models is briefly described including the possible role of apoptosis. The motivation to explore possible neuroprotection by apoptosis inhibitors is explained. The need for diagnosing Parkinson's disease in an early phase will become necessary if neuroprotective treatment really becomes available.
The role of presynaptic striatal imaging in the diagnostic work-up of Parkinson's disease in an early stage by means of F-DOPA PET and FP-CIT SPECT is described.

Chapter 2 gives an extensive description of Parkinson's disease. Epidemiology, including mortality, regional, racial and sexual variation is described and sub classifications of Parkinson's disease. An overview of motor and non-motor symptoms is given, followed by pathology findings. Several other movement disorders in the elderly and changes in gait with aging are described.
Functional Neurobiology of Aging; Chapter 68. Edited by Hof and Mobbs.

Chapter 3 describes the correlation between motor behaviour and F-DOPA uptake in a well known animal model for Parkinson's disease: the MPTP-treated monkey. Eight rhesus-monkeys received MPTP infusions and became parkinsonian to various degrees. Motor signs were rated regularly quantitatively and qualitatively and correlated with striatal uptake of [^{18}F]-DOPA as measured with PET. *Submitted.*

In **chapter 4** the neuroprotective effect of the compound TCH346 is evaluated in MPTP-treated monkeys. Eight rhesus monkeys received MPTP-infusions bilaterally by a two-step procedure, which induces a stable parkinsonian animal model. The effects of TCH346 on behaviour and striatal dopaminergic integrity were evaluated by means of quantitative and qualitative analyses of behaviour and striatal F-DOPA uptake. Post-mortem immunohistochemical analyses of the brains were performed. *Neurobiol Dis 2003;14:205-17.*

Chapter 5 describes the correlation between two radiotracers: FP-CIT SPECT and F-DOPA PET in patients with different stages of Parkinson's disease. Also correlation between uptake of both tracers and motor scores are established and the ability to distinguish patients with advanced disease from de novo patients is described. In this study 13 de novo parkinsonian patients and 17 patients with advanced stage of disease underwent both FP-CIT SPECT scans and F-DOPA PET scans. *Eur J Nucl Med Mol Imaging 2006;33:200-9.*

Chapter 6 focuses on the ability of FP-CIT SPECT and F-DOPA PET to distinguish de novo parkinsonian patients from the healthy population and establishes the sensitivity and specificity of both radiotracers in this. Ten healthy volunteers received a FP-CIT SPECT scan, 10 healthy volunteers an F-DOPA PET scan and a total of 28 parkinsonian patients had both a FP-CIT SPECT and a F-DOPA PET scan. *Eur J Nucl Med Mol Imaging 2009;36:454-62.*

Chapter 7 contains the summary of this thesis.

In **chapter 8** discussion and future perspectives are discussed.

A summary of this thesis in Dutch including future perspectives is given in **chapter 9**.

In **chapter 10** a list of abbreviations can be found.

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